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# Production of High Quality Functional Labneh Cheese Using Exopolysaccharides Producing Encapsulated *Lactobacillus Bulgaricus*

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**Abstract:** Production of polysaccharides by lactic acid bacteria is vital and its technology is very important, too in dairy study. The selection of expolysaccharides producing encapsulated *Lactobacillus bulgaricus* in alginate gel and the factors affecting the production of polysaccharides such as time, temperature, pH and the growth with *Streptococcus thermophilus* were studied. pH 5.5 and temperature 35 ℃/18hr were found to be optimal for exopolysaccharides production by encapsulated *L. bulgaricus* and free culture yielding 600 mg/L and 380 mg/L respectively. Co-culturing *L. bulgaricus* with *S. thermophilus* increased exopolysaccharides production at 35 ℃/18hr to 660 mg/L by encapsulated cells and 400 mg/L by free culture. Results of this study were applied in the manufacture of Labneh cheese vitally containing probiotic species of *L. acidophilus, L. gasseri*, *L. johnsonii* and protection of these bacteria by polysaccharide produced encapsulated *L. bulgaricus*. The use of exopolysaccharides producing encapsulated *L. bulgaricus* and probiotic bacteria can provide acceptable quality to functional Labneh. This product can be used as therapeutic and diabetic milk product with highly acceptable qualities. It is noted that all of the results are the means of triplicate experiments.

**Key words:** *Lactobacillus bulgaricus*, encapsulation, Labneh, *Sterptococcus thermophilus*, polysaccharides, probiotic bacteria.

## 1. Introduction

Most Lactic acid bacteria (LAB) producing Exopolysaccharides (EPS) belong to the genera *Streptococcus*, *Lactobacillus*, *Lactococcus*, and *Pe-dicococcus*. Also, some strains of genus *Bifidobacterium* are able to produce these biopolymers [1].

Some strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* produce a ropy exopolysaccharide substance. This mucoid substance increases the viscosity of yogurt while concurrently decreasing whey separation [2]. Exopolysaccharide from *L. bulgaricus* are heteropolysacharides, consisting of repeating units of monomers, such as glucose, galactose and rhamnose. Other strains are able to produce phosphopolysaccharides. A general feature of *L. bulgaricus* is the production of two different molecular weight EPS fractions [3, 4].

Incorporation of (EPS) or EPS-producing (EPS+) cultures in dairy foods can provide viscosifying, stabilizing, and water-binding functions. EPS also contributes to the mouth-feel, texture, and taste perception. They play an important role in the manufacturing of fermented dairy products such as yogurts, drinking yogurts, cheeses, fermented creams and milk-based desserts. Additionally, they have been claimed to properties beneficial to health as prebiotics or due to their antitumor, antiulcer, immunomodulating or cholesterol-lowering activities and positively affect gut microflora [5, 6].

Several studies highlighted the positive effect of exopolysaccharides (EPS) producing cultures on the physical and functional properties of reduced-fat dairy

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products such as a fat free acid-coagulated soft cheese [7], reduced-fat Cheddar cheese [8]. Also exopolysaccharide (EPS) producing starter cultures are used to improve the physical properties of yogurt [2, 9].

Different methods (physical entrapment in polymeric networks, attachment or adsorption to a performed carrier, membrane entrapment, and microencapsulation) have been used for immobilizing LAB. The purpose of these techniques is either to retain high cell concentrations within the bioreactor, or to protect cells from a hostile environment [10, 11]. Other advantages of Immobilization Cell Technology (ICT) are the reuse of cells, protection against shear damage and the high biological stability during long-term continuous fermentation [3, 12].

Few works used ICT for produced polysaccharides such as Refs. [13, 14] which studied the production of exopolysaccharides (EPS) by *L. rhamnosus* RW-9595M during pH-controlled batch cultures with free cells and repeated-batch cultures with cells immobilized on solid porous supports. The study clearly shows the high potential of the strain *L. rhamnosus* RW-9595M and immobilized cell technology for production of EPS as a functional food ingredient. Also, M.D.A. El-Ahwany [15] used optimized conditions with cells immobilized by using agar and alginate gel materials for xylanase production by *Bacillus pumilus*. They found that immobilized cells in 2% alginate showed a 2.6-fold increase in xylanase specific activity compared to free cells.

The objectives of the present work were to determine the optimum temperature, time and pH for production of EPS by *Lactobacillus bulgaricus* ropy strains as culture and microcapsulated to produce new symbiotic Labneh.

## 2. Materials and Methods

### *2.1 Source of Cultures*

*Streptococcus thermophilus* and *Lactobacillus acidophilus* were obtained from Chr. Hansen's Lab., Denmark, *Lactobacillus bulgaricus* ropy strain obtained from Dairy Microbiology Lab. National Research Center, Dokki, Cairo, Egypt [16]. *Lactobacillus gasseri* B-2178 and *Lactobacillus johnsonii* B-14168 were donated by (NRRL) Illinois, USA.

### *2.2 Screening Polysaccharides Producing L. Bulgaricus Strains*

Active culture of *L. bulgaricus* was spoiled (5 ml) on the surface layer of MRS agar supplemented by 50 gm sucrose. The plates were incubated under anaerobic conditions at 37 ℃/72hr. At the end of incubation time the colonies were tested for ropiness by touching them with sterile inoculation loop [17].

### *2.3 Preparation of Culture*

*Lactobacillus* strain and *S. thermophilus* were activated in skim milk 11% and incubated at 35 ℃/18hr.

### *2.4 Preparation of Micro-capsulation (Beads)*

MRS broth was used to yield *L. delbreuckii* ssp. *bulgaricus* (ropy Strain) cells for preparation, of microcapsulation. The broth was inoculated with 2% active *L. delbreuckii* ssp. *bulgaricus* culture and incubated at 37 ℃ for 24 hr. Cells were harvested by centrifugation at 5,000 rpm for 15 min at 4 ℃ and cells biomass were washed twice with saline and used to prepare beads.

Suspension cells was mixed with an equal volume of sodium alginate (4%) the mixture was added drop wise into solution of sodium chloride (0.2 mol/L) and calcium chloride (0.5 mol/L) which was magnetically stirred at 200 rpm. Tell them alginate beads were formed [18].

### *2.5 Optimization Condition of Exopolysaccharides Production by Culture and Beads of L. Bulgaricus*

Skim milk powder 10% w/v was inoculated by 2% *L. bulgaricus* culture and beads to study the production of polysaccharide. According to B. Zisu & P. Shah [19], the inoculated milk was incubated at 37 ℃ for 48 hr and sampled at 0, 6, 12, 24, 36 and 48 hr to study the

time effect on producing of polysaccharide. Also to study the effect of temperature the inoculated milk was incubated at 30, 35, and 40 ℃/18hr. The pH of sterilized milk was adjusted to pH 4.5, 5.4 and 6.8 by 10% satirized lactic acid (w/v) and was inoculated by *L. bulgaricus* (culture and beads) 2% then incubated at 35 ℃/18hr to study the effect of pH. The count of *L. bulgaricus*, the concentration of EPS and viscosity were determined in all samples [19].

*2.6 Effect of Addition of S. Thermophilus with L. Bulgaricus as Yoghurt Culture on Production of EPS*

Incubation at 35 ℃/18hr the combination of *S. thermophilus* with *L. bulgaricus* as yoghurt culture in skim milk powder 10% (w/v) was used as the following *S. thermophilus* with *L. bulgaricus* non ropy strain culture (NRSC), *S. thermophilus* with *L. bulgaricus* ropy strain culture (RSC) and *S. thermophilus* with encapsulated *L. bulgaricus* ropy strain (RSM) for measuring the EPS production.

*2.7 Labneh Manufacture*

3 kg of cow milk and 3 kg of buffalos milk (from Cairo University, Department of Dairy Science) were homogenized at 35 ℃ in RANNIE, Copenhagen. Homogenized milk was heated to 90 ℃/20 min thin cold to 35 ℃. Milk was divided to two equal parts. The first part was inoculated with mixed culture of *S. thermophilus* and *L. bulgaricus* ropy strain culture (RSC) 2% v/v 1:1. The second part was inculated with mixed culture of *S. thermophilus* and encapsulated (beads) *L. bulgaricus* ropy strain (RSM) 2% v/v 1:1. Each part was divided to three portions to supplement by probiotic 0.5%, *L. acidophilus*, *L. gasseri* and *L. johnsonii* 0.5%.

The treatments of Labneh were as the following:

Treatment I (RSC) + *L. acidophilus*

Treatment II (RSC) + *L. gasseri*

Treatment III (RSC) + *L. johnsonii*

Treatment V (RSM) + *L. acidophilus*

Treatment IV (RSM) + *L. gasseri*

Treatment IIV (RSM) + *L. johnsonii*

All treatments were incubated at 35 ℃/18 hr. Then cooled overnight at 4 ℃, mixed thoroughly with 1% (w/w) of sodium chloride put into cheese cloth bags and then hung in the refrigerator for 12 hr to allow whey drainage. The fresh Labneh was filled into 50 gm plastic containers and kept at 5 ℃ and sampled at 0, 5, 10 and 15 days for microbiological analysis but chemical and sensory analysis was at fresh and after 15 days [20].

*2.8 Isolation and Quantification of EPS*

The samples were collected and frozen until measuring the EPS according to Ref. [19]. 100 ml of the frozen sample was thawed overnight at 4 ℃ to which 20 ml of 20% (w/v) trichloroacetic acid solution (Sigma) was added to precipitate casein followed by centrifugation at 10,000×g for 20 min at 4 ℃ to remove the precipitate and bacteria cells. The supernatant was neutralized to pH6.8 with 4 M NaOH and boiled for 30 min in a water bath and re-centrifuged (10,000×g for 20 min at 4 ℃).

An equal volume of chilled ethanol was added to the supernatant to precipitate (EPS) from solution and agitated at 100 rpm and 4 ℃ overnight. The crude EPS pellet was recovered by centrifugation (10,000×g for 20 min at 4 ℃). The EPS was quantified using the phenol-sulfuric method [21].

*2.9 Viscosity Measurement*

A coaxial cylinder viscometer (Buhlin V88, Sweeden) equipped with C30 system which permits a gap of 1.5 mm between the two cylinders and attached a workstation loaded with V88 viscometer programmer was used. The measuring system was filled with about 40 ml of the sample at 25 ℃. Measurement of viscosity was carried out at share rate between 1.25-9.77 1/S at 3 min intervals [22].

*2.10 Chemical Analysis*

The total solids and fat content were measuring by

methods described in Ref. [23]. pH value was measured using a digital pH-meter with combined glass-Calomel electrode (HANNA). Protein content was determined according to Ref. [24]. Acetaldhyde and diacetyle were determined according to Ref. [25].

*2.11 Microbiologicl Analysis*

Labneh cheese samples were analyzed for *Lactobacillus* count on MRS agar (Oxoid). The count of *L. bulgaricus* in beads was determined by using buffer phosphate (pH7.2) in first dilution. According to Ref. [26], M17 (Oxoid) was used to determine *S. thermophilus*.

*2.12 Sensory Evaluation*

The organaleptic properties of Labneh were assessed by 20 panelists of the experienced staff members of Dairy Department National Research Center. Samples were evaluated for flaver (50 points), body and texture (40 points) and appearance (10 points) [27].

*2.13 Statistical Analysis*

Statistical analysis was performed using the GLM procedure with software [28]. Duncan's multiple comparison procedure was used to compare the means. A Probability of $P \geq 0.05$ was used to establish statistical significance.

## 3. Results and Discussion

*3.1 Optimum Conditions for the EPS Production by Culture and Beads of L. Bulgaricus*

3.1.1 Effect of Time

Fig. 1A clears the EPS yield by *L. bulgaricus* (RSC and RSM) during the fermentation time 48h. It began produce after 6 hr by both treatments. The highest yield was collected at 18hr (276 mg/L for culture (RSC) and 317 mg/L for microencapsulation RSM). On other contrary, the amount of EPS production declined after the first 18 hr. The decline in the EPS content observed at 24 hr (216 mg/L and 262 mg/L) and at 48 hr of fermentation (191 mg/L and 242 mg/L) for culture and encapsulated cultures respectively. These results may due to enzymes degradation [19]. While T.Y. Lin [29] found that the highest EPS yield from *L. helveticus* BCRC14030 was730 mg/L which observed at the less favorable fermentation condition of 60 h with a total viable count, $1.9 \times 10^7$ cfu./ml.

The determination of the viscosities during milk fermentation was appeared in Fig. 1B, The increase in apparent viscosity in the fermented milk by encapsulated culture was observed at 6hr and derived to maximum at 18hr likeness with the production of EPS and began to decline after this time. The difference in apparent viscosities was not clear with RSC fermentation by ropy strain. These results were agreed by B.G. Zeynep [30], who reported that yogurts made with ropy cultures (polysaccharide production) had higher viscosity values than yogurts made with no ropy cultures.

The cell growth was accelerated over the first 6 h as shown in Fig. 1C and reached to maximum concentration after 18hr. They could be reached to 8.4 log cycle for culture, and 10.9 log cycles for cells in encapsulated culture but free cell arrived to 7.3 log cycle at 18 hr. our results coincide with those stated by Refs. [13, 15].

3.1.2 Effect of pH

Fig. 2A illustrate the role of initial pH of milk on production of EPS after 18 h at 35 ℃. At pH5.5, EPS production was favored by *L. bulgaricus* yielding 366 mg/L by (RSC) and 503 mg/L by RSM. Our results near to B. Zisu & M. Svensson [19, 31] who found that some strains from *S. thermophilus*, produced more EPS than the other strains at pH4.5 in enriched milk. In spite of this pH created unfavorable growth conditions for the *S. thermophillus*.

Data presented in Fig. 2B show the viscosity of fermented milk at different initial pH. The highest viscosity was observed at pH5.5 for both RSC and RSM. Also the encapsulated cells were give high viscosity in different treatment. F. Vaningelgem [32] measured the viscosities for some strains of *S.*

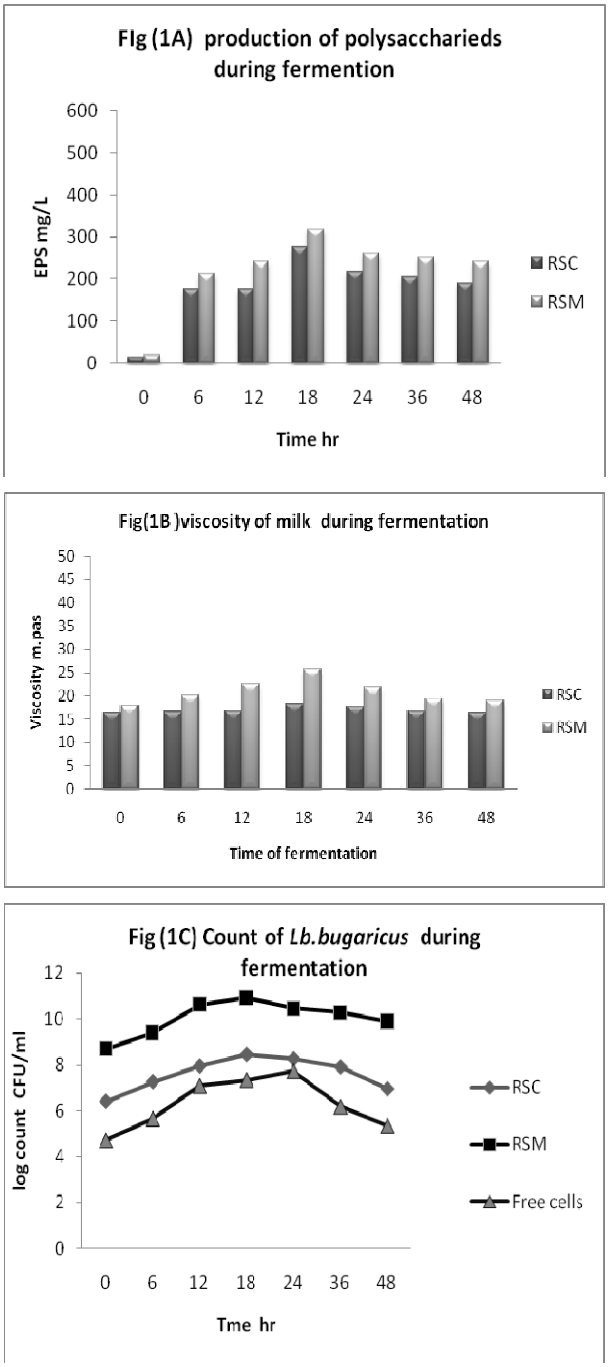



**Fig. 1 Effect of time on EPS production by free culture RSC or encapsulated culture *L. bulgaricus* RSM during fermentation at 35 ℃/48hr.**

*thermophilus* at a spindle speed of 50 rpm. They observed the apparent viscosity increased when the pH dropped below 5.75 during the fermentation. These results could be agreement with count of *L. bulgaricus* which clear in Fig. 2C. At initial pH4.5 both cell numbers of two treatments were lower than another

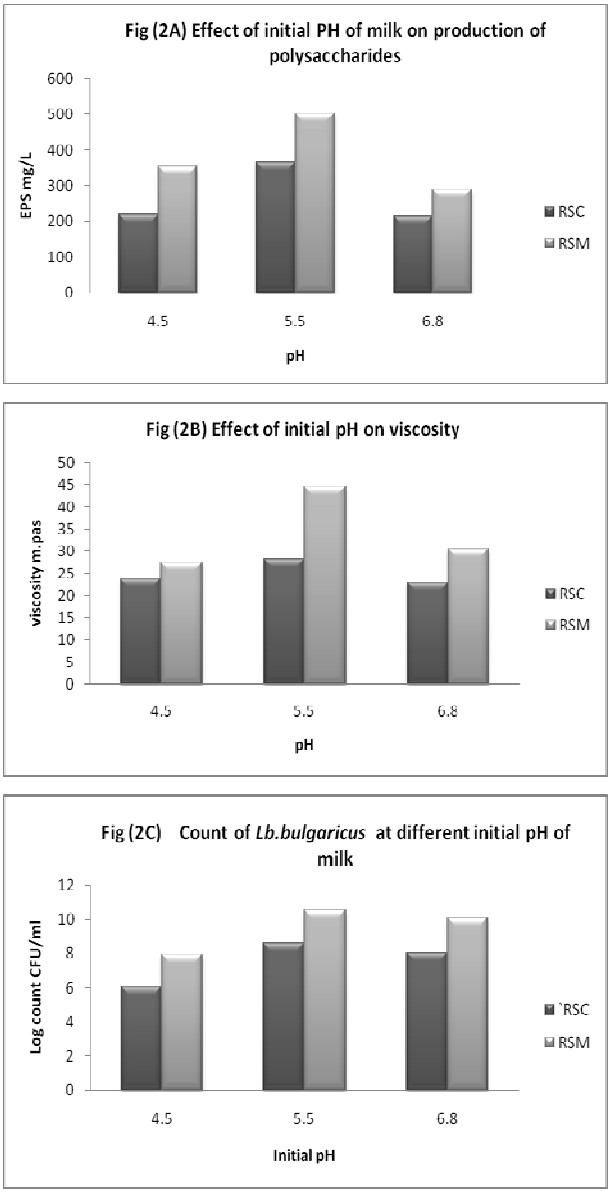



**Fig. 2 Effect of initial pH of milk on EPS produced by free culture RSC or encapsulated culture RSM *L. bulgaricus*.**

initial pH. We observed at pH5.5 the cells density in beads were yielded over 10.5 log cycle cfu./ml, and count of cells culture were over 8.6 log cycle cfu./ml fermented milk. The data is similar with Refs. [33, 19].

3.1.3 Effect of Temperature

Fig. 3A shows the effects of temperature on the EPS production by *L. bulgaricus* RSC and RSM which was grown in milk at 30, 35 and 40 ℃. The optimum temperature for EPS production was 35 ℃, yielding 327 and 695 mg/L for culture and encapsulated culture respectively after18 hr of fermentation.

The viscosity of fermented milk at different temperature was cleared up at Fig. 3B. At both temperature 35 and 40 ℃ the viscosity increased, but at 30 ℃ show decline in viscosity by *L. bulgaricus* RSC and RSM. The cell counts of *L. bulgaricus* for both RSC and RSM were appearance in Fig. 3C. No substantial different observed in count at 35 and 40 ℃ in culture and also in beads. Our results near to Refs. [19, 34].

From the previous data, the highest yield from EPS production by *L. bulgaricu* RSM has associated to growth at optimum temperature 35 ℃, pH5.5 and after 18hr to produce highest yield of EPS than *L. bulgaricu* RSC. The data were agreement with M.D.A. El-Ahwany [15] who observed a 2.6 fold increased in xylanase produced by *B. pumilus* with immobilized cells than free cells at optimize medium condition.

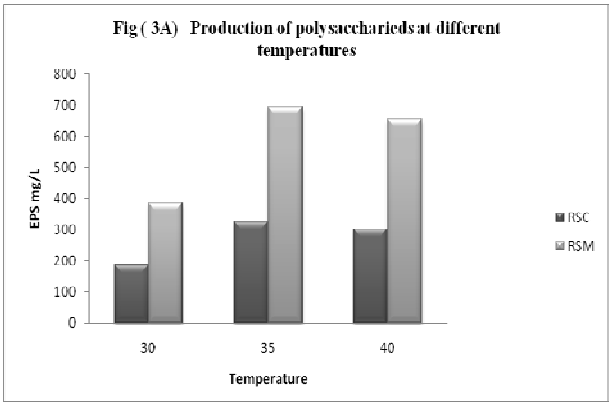
### *3.2 Effect of Co-culturing L. Bulgaricus RSC and RSM with S. Thermophilus (Yoghurt Culture) on EPS Production*

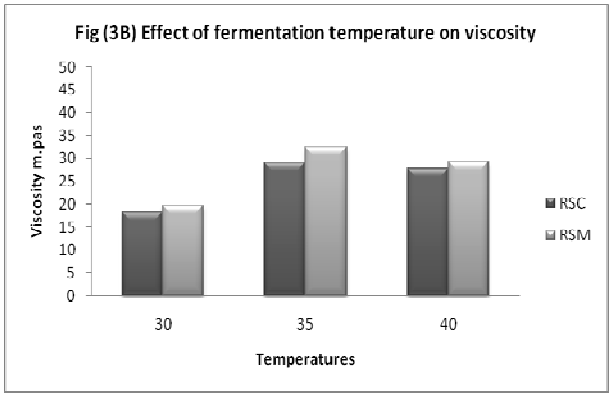
Effects of fermentation with addition of *S. thermophilus* on production of EPS are shown in Fig. 4. The presence of *S. thermophilus* showed a clear increase in EPS production after18 hr at 35 ℃ with *L. bulgaricus* RSC and RSM. The quantities of EPS increased by ~50 mg/L in *L. bulgaricus* RSC treatment but with RSM they become greater than the twice of EPS produced by *L. bulgaricus* RSM only. This due to the synergistic interrelation between *L. bulgaricus* and *S. thermophilus*.

### *3.3 Characteristics of Functional Labneh Manufactured by Encapsulaed L. Bulgaricus (Ropy Strain)*

#### 3.3.1 Survival of Lactobacillus

Fig. 5 shows the viability of lactobacillus strains *L. acidophilus*, *L. gasseri* and *L. johnsonii* as total count of lactobacillus in Labneh manufactured by *L. bulgaricus* RSC and RSM during the storage at refrigerator. The count decreased approximately 0.8 logarithmic cycle cfu/gm after 15 days in RSC treatments with all lactobacilli. While, the lactobacillus



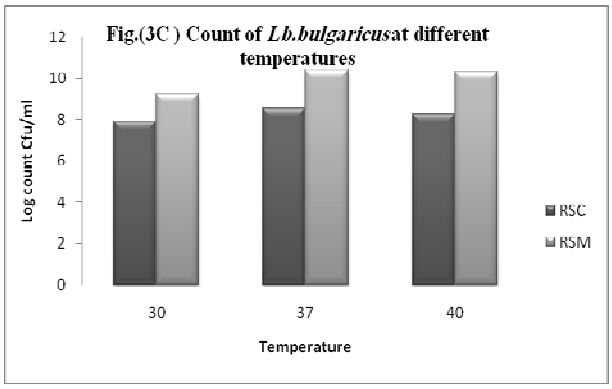




**Fig. 3 Effect of incubation temperatures/18 hr on production of polysaccharide by free culture RSC or encapsulated culture RSM *L. bulgaricus*.**

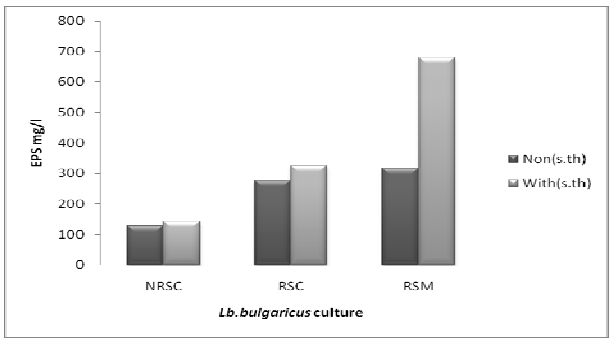



**Fig. 4 Exopolysaccharides produced by ropy or no ropy strain of *L. bulgaricus* in co-culture with *S. thermophilus*.**

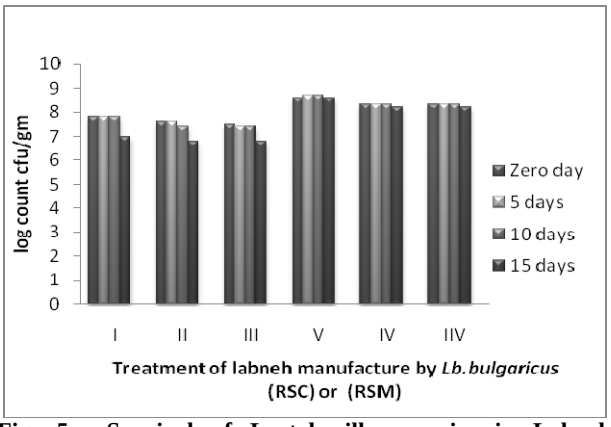



**Fig. 5 Survival of Lactobacillus species in Labneh manufactured by culture or encapsulated *L. bulgaricus*.**

survival was kept up firm at 8.6, 8.3, and 8.3 logarithmic cycle cfu/gm for three strains treatments respectively encapsulated beads treatments after 15 days at refrigerator. We observed that the density of cells of *L. bulgaricus* in beads was low and stabile at approximately 8.6, 8.3 and 8.3 logarithmic cycle cfu/gm in three treatments through storage at refrigerator. C.P. Champagne [35] observed an increase in free-cell density as the beads were used for successive fermentations. The number of free cells in the fermented milks were initially lower when low-cell-density beads were used. The free-cell population accounted for 2.4% of the total cell count in the bioreactor. So, it was obvious that the majority of populations are from probiotic lactobacillus in the Labneh treatments.

3.3.2 Chemical Composition of Labneh

The mean chemical composition of cheese is presented in Table 1. Slight changes were observed in pH, protein and lactose in chesses treatments during the storage for 15 days. The total solid of cheese treatments containing RSM was lower than that containing RSC only. There are beside to increased in EPS containing at the end of storage ≥ 30 mg/L in Labneh manufacture by RSM as shown in Fig. 6. This was due to the ability of EPS to produce cheese with high water holding capacity. N.H. Ahmed [7] mentioned that the moisture and yield were about 2% higher in the cheese made with the EPS-producing culture than that made by EPS-nonproducing culture, and about 4% than the control.

As shown in Figs. 7A & 7B, Diacetyl and Acetaldehyde decreased after 15 days of storage in the all treatments of Labneh. The acetaldehyde content of Labneh treatments made by RSM was more than that made with RSC. These results could be attributed to increment in metabolic activity for lactobacillus strains in this treatment. Also, there were no significant differences between all samples of Labneh during storage in the level of diacetyl and Acetaldhyde. These results are in agreement with those reported by R.M. Badaw [36].

3.3.3 Sensory Evaluation

Sensory evaluation of Labneh produced from *L. bulgaricus* RSC and RSM with different beneficial lactobacillus strains (treatments 1-6) are depicted in

**Table 1 Chemical composition of Labneh manufactured by *L. bulgaricus* RSC or RSM with lactobacillus probiotic strains during storage.**

| Chemical analysis | RSC in Labneh with | | | | | | RSM in Labneh with | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *L. acidophilus* | | *L. gasseri* | | *L. johnsonii* | | *L. acidophilus* | | *L. gasseri* | | *L. johnsonii* | |
| | fresh | 15 days | fresh | 15 days | fresh | 15 days | fresh | 15 days | fresh | 15 days | fresh | 15 days |
| pH | 4.82 | 4.58* | 4.82 | 4.45* | 4.6 | 3.35* | 4.93 | 4.35* | 4.92 | 4.33* | 4.94 | 4.38* |
| Fat% | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 | 7.0 |
| T.S% | 30.5 | 31.45* | 29.42 | 30.96* | 30.93 | 32.88 | 27.83 | 28.37* | 27.97 | 29.08* | 29.37 | 29.56* |
| Protein% | 10.4 | 10.4* | 11.0 | 11.02* | 11.65 | 10.97 | 10.78 | 10.63* | 11.o2 | 10.97 | 10.7 | 10.42 |
| Lactose% | 4.05 | 4.00* | 4.2 | 4.15* | 4.3 | 4.25 | 4.0 | 4.0* | 4.3 | 4.18 | 4.3 | 4.23 |
| EPS mg/L | 407 | 411 | 545 | 557 | 445 | 481 | 435 | 440 | 570 | 579 | 485 | 490 |

* Significant differences.

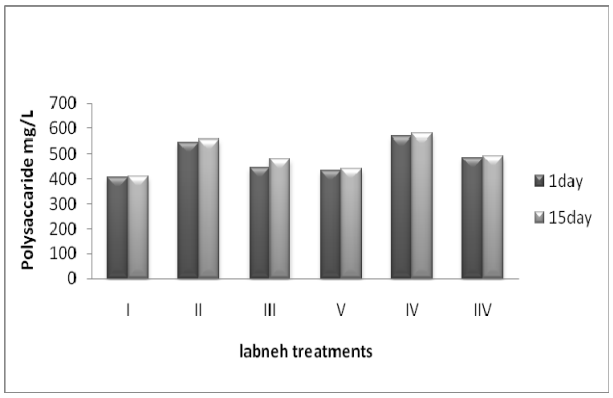



**Fig. 6 Polysaccharides produced in Labneh cheese by culture and encapsulated *L. bulgaricus*.**

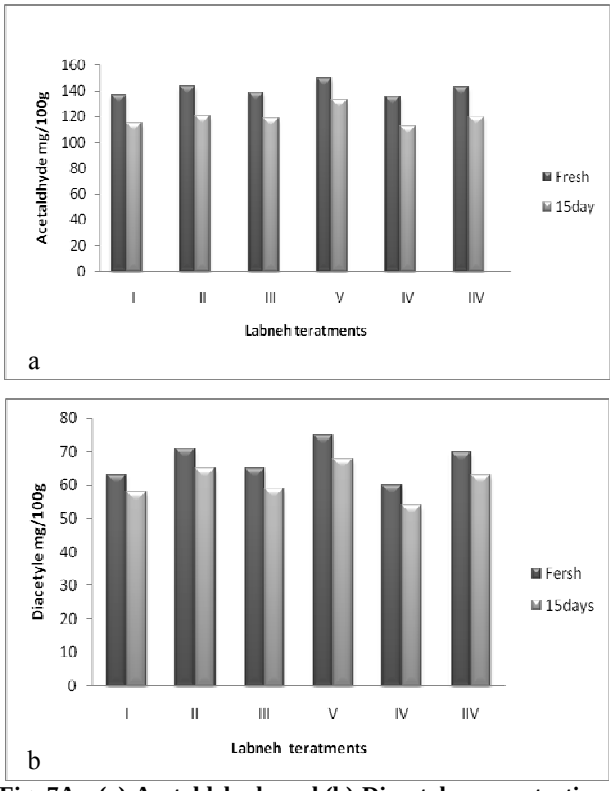



**Fig. 7A (a) Acetaldehyde and (b) Diacetyle concentrations in fresh Labneh and after 15 days.**

Fig. 8. The results indicated with all Labneh treatments had a good quality, but we observed the treatment with RSM was ranked higher scores for body & texture than other treatment. This could be attributed to interact exopolysaccharides produced by bacteria with the free water in the gel-like structure, so, the texture is improved. Also, N. Dabour [8] highlighted the positive effect of EPS producing culture on the physical and functional properties of reduced fat cheese.

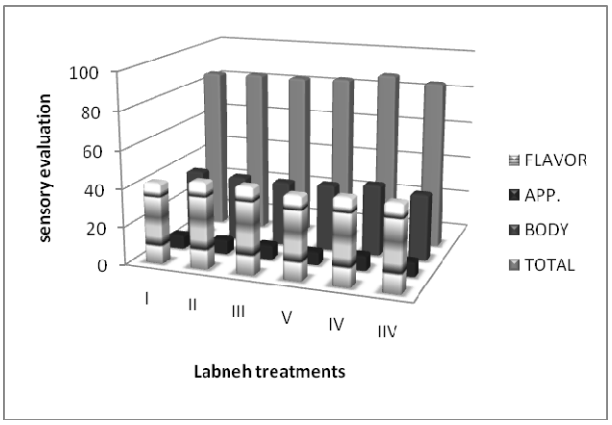



**Fig. 8 Sensory evaluation of Labneh manufactured by ropy strain culture or encapsulated *L. bulgaricus*.**

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